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New Rodent Population Models May Inform Human Health Risk Assessment and Identification of Genetic Susceptibility to Environmental Exposures

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Background: This paper provides an introduction for environmental health scientists to emerging population-based rodent resources. Mouse reference populations provide an opportunity to model environmental exposures and gene–environment interactions in human disease and to inform human health risk assessment.

Objectives: This review will describe several mouse populations for toxicity assessment, including older models such as the Mouse Diversity Panel (MDP), and newer models that include the Collaborative Cross (CC) and Diversity Outbred (DO) models.

Methods: This review will outline the features of the MDP, CC, and DO mouse models and will discuss published case studies investigating the use of these mouse population resources in each step of the risk assessment paradigm.

Discussion: These unique resources have the potential to be powerful tools for generating hypotheses related to gene–environment interplay in human disease, performing controlled exposure studies to understand the differential responses in humans for susceptibility or resistance to environmental exposures, and identifying gene variants that influence sensitivity to toxicity and disease states.

Conclusions: These new resources offer substantial advances to classical toxicity testing paradigms by including genetically sensitive individuals that may inform toxicity risks for sensitive subpopulations. Both *in vivo* and complementary *in vitro* resources provide platforms with which to reduce uncertainty by providing population-level data around biological variability. https://doi.org/10.1289/EHP1274

Introduction

Environmental scientists worldwide are tasked with assessing health risks of environmental exposures to chemicals, and the methodology used to assess risks is continually evolving. Chemical risk assessments involve evaluation of exposure and prediction of health risks and outcomes with the goal to inform decision making to control or otherwise respond to environmental hazards. Biological variability is an important factor in defining human responses to chemical exposures and variability—of various etiologies—can contribute to whether an individual is susceptible or resistant to an adverse outcome. Variation in response to chemicals is determined by both extrinsic (e.g., co-morbidities, exposure dose concentration, co-exposures, nutrition, and psychosocial stressors) and intrinsic (e.g., genetic sequence or epigenetic variation, age/life stage, sex) factors (Zeise et al. 2013).

Because assessment of risk inherently involves variability in responses, the methods used in risk assessment must be designed to quantitatively address population variation with precision. Uncertainties exist, in the estimation of exposures, the identification and measurement of health effects associated with exposures, and the methodologies used to assess and characterize population and occupational risks. The current risk assessment paradigm utilizes a standardized uncertainty or threshold factor to account for variability in cases where population-based data are unavailable (U.S. EPA 2014). As methods for assessing population health risks evolve, an emerging idea is to actively consider multiple determinants of population health and their interactions prior to the design of testing strategies. Advances in molecular methods and an interest in pathway-based risk assessments have driven interest in development of a next generation "NextGen" framework or risk assessment (Cote et al. 2016).

Advanced risk assessment techniques, a population health approach, and the NextGen framework for risk science are crucial elements for the protection of particularly sensitive individuals within exposed populations. Indeed, the challenges and opportunities to address human variability in NextGen risk assessments was recently reviewed extensively by Zeise and coauthors (Zeise et al. 2013). Furthermore, the benefits of understanding gene–environment connections and understanding their implication for risk management has been argued to be an integral component of NextGen risk strategies (Krewski et al. 2014).

To implement this new strategy, new tools are necessary for assessing population-level responses for which the determinants of health outcomes can be multifactorial. Genetic variation contributes substantially to interindividual differences in susceptibility to toxicant-induced adverse health events (Collins et al. 2016). Recent data suggest that an improved understanding of the genetic variability of toxicant responses will enable more accurate chemical toxicity assessments, and methods to enable use of population-level data to predict toxicity risks are an area of active investigation (Eduati et al. 2015). Development of a variety of tools is necessary to identify the range of human responses to chemical hazards.

Epidemiological studies in humans are costly, time consuming, and often confounded by other factors such as age, co-morbidities, and exposures to a wide variety of chemical exposures over the lifetime of the individual. Studies of human populations will always provide the strongest causal evidence of toxicity when they are available, but when they are not, the use of genetically diverse mouse populations can be a powerful tool to model human population responses. When human evidence is limited, experimental models can identify sensitivities of relevance to

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humans and support or further characterize findings in human populations, strengthening the biological plausibility of the evidence in humans. Rodent population resources, particularly involving the mouse, are being developed and are increasingly well characterized for numerous traits. An advantage of this approach is the inclusion of both genetically sensitive and resistant mice that may react more "like" humans at a given dose than would be possible in a conventional study using a single rodent strain or classical outbred stock. Newly available mouse populations, as will be discussed in this review, harbor genetic variation that is comparable to the human population, but with an advantage that variation is more randomized than in humans, meaning more information about variability can be obtained in a smaller cohort of mice than would be needed for a human epidemiological study.

To explore opportunities for the use of the population-based rodent models in the context of environmental health science research, the National Institute for Environmental Health Sciences held a workshop on the topic of "population-based rodent resources for environmental health sciences." What follows is not a summary of the meeting; however, the authors are indebted to the workshop's many excellent speakers for guiding thinking around key concepts in the present manuscript. This review will attempt to describe these population-based rodent resources and their potential uses within the risk assessment framework.

Current Rodent Models Utilized for Chemical Toxicology Studies

Classical toxicological testing in animal models has traditionally been conducted in rodent species; however, rodent strains that have been classically used contain no genetic diversity because they were intentionally inbred to fix the genome and reduce variability in measurements. In the case of the National Toxicology Program (NTP), the preferred rodent models are the B6C3F1 mouse (an F1 hybrid cross between C57BL/6 and C3H inbred mouse strains) and the Fisher 344 (F344) inbred rat. Rodents are inbred via brother–sister mating for at least 20 generations to fix the genome. Owing to extensive historical pathology data from studies using these rodents, B6C3F1 mice and F344 rats can thus be thought of as defined reagents. There are advantages to standardization of genetic context—in this case, reproducibility is achieved because B6C3F1 mice and F344 rats are the equivalents of genetic clones, in which each individual is genetically the same (isogenic). Because of this isogenicity, data are largely reproducible across experiments, although epigenetic modifiers and environmental variables may affect experimental outcomes to varying extents. A reasonable argument for using inbred rodent strains is extensive historical experience with these models, which provides a context for comparing data across chemicals and a knowledge base of background lesions that informs pathological determination of chemical response. Historical databases built on conventional rodent strain data conveniently enable cross-study comparisons. There are also advantages in terms of low variability within quantitative dose–response curves that can be generated using these traditional resources.

Although the F344/N rat has been used for the rodent bioassay by NTP for over 30 y, this strain has developed many health and reproductive issues over time. The NTP convened a workshop in 2005 entitled "*Animal Models for the NTP Cancer Bioassay: Strains and Stocks—Should We Switch?*" to explore the use of alternative rat models for toxicity studies. Based on the recommendations from this workshop, NTP made the decision to focus on the use of the Envigo Hsd:Sprague-Dawley (SD) rat as the primary rat model for initially evaluating a test compound or chemical. The SD rat is outbred, which affords some genetic diversity, and it is also one of the most commonly used rat stocks in pharmaceutical testing.

Many investigators assume that conventional outbred stocks, such as SD rats, reflect substantial genetic diversity. SD rats, like most outbred stocks, are genetically undefined in that each animal is genetically distinct and labile in terms of genetic changes based on random genetic drift (Festing 2014). Several reports have suggested that the use of classical outbred stocks makes many toxicology experiments less sensitive and complicates assessment of genetic from nongenetic variation in contributing to the toxicity outcome (Festing 2016). Specifically, classical outbred stocks such as SD rats are thought to add more experimental noise in terms of spontaneous background findings, potentially complicating estimation of quantitative characteristics. However, a tradeoff is a greater ability to detect chemical hazards that might otherwise be missed in a single inbred strain. Inbred strains are more stable, better defined, and have more extensive background pathology data in archives as compared with classical outbred stocks. Thus, toxicity screening designs have been suggested that use small numbers of inbred animals of several mouse strains together. This approach would reveal genetic variation that is not seen when using a single (conventional) outbred stock and decisions could be based on either the most susceptible strains alone or on the population as a whole.

Another major drawback of the single-strain approach is that mice or rats with different genetic backgrounds may be more or less sensitive to the adverse health effects of the test chemical. In fact, many recent studies have shown that genetic background can have a major effect on toxicity outcomes and on the ability to detect human-relevant adverse effects. For example, in a recent study that exposed 35 different inbred strains of mice (along with B6C3F1/J mice) to acetaminophen, it was found that some mouse strains are resistant to acetaminophen-induced hepatotoxicity, whereas other strains sustain upwards of 80% liver necrosis at the same dose level (with B6C3F1 mice falling at the high end of sensitivity) (Harrill et al. 2009b). Similar investigations have found that genetic background affects adverse outcomes of exposures to environmental contaminants, such as liver toxicity associated with 2,3,7,8-tetrabromodibenzo-*p*-dioxin (TBDD) (Nguyen et al. 2016) and renal toxicity induced by trichloroethylene (Yoo et al. 2015).

Description of Different Population Models

Mouse Diversity Panel

A mouse diversity panel (MDP) is a common and straightforward method for measuring responses across a genetically diverse population. MDPs are comprised of approximately 20–40 inbred strains of *Mus musculus (M. m.) domesticus* and *M. m. musculus* subspecific origin, typically inclusive of commonly utilized laboratory strains such as C57BL/6J and Balb/cJ, although the exact strain composition may vary (Figure 1). A related resource, the Mouse Hybrid Diversity Panel (MHDP), is comprised of 30 classical inbred strains and approximately 70 recombinant inbred (RI) strains primarily derived from crossing C57BL/6J × DBA/2J (B × D strains) and A/J × C57BL/6J (A × B and B × A RI strains) (Lusis et al. 2016). A benefit of these resources is that mice within a given strain are genetically identical and reproducible. However, a major drawback is that many classical strains are closely related genetically due to their derivation from common ancestor strains or stocks. Thus, strain selection becomes important to maximize genetic diversity across the panel of strains surveyed, and genetic variation among classical strains is markedly lower than that present in rationally designed RI strain panels. Despite these limitations, MDP studies have yielded important

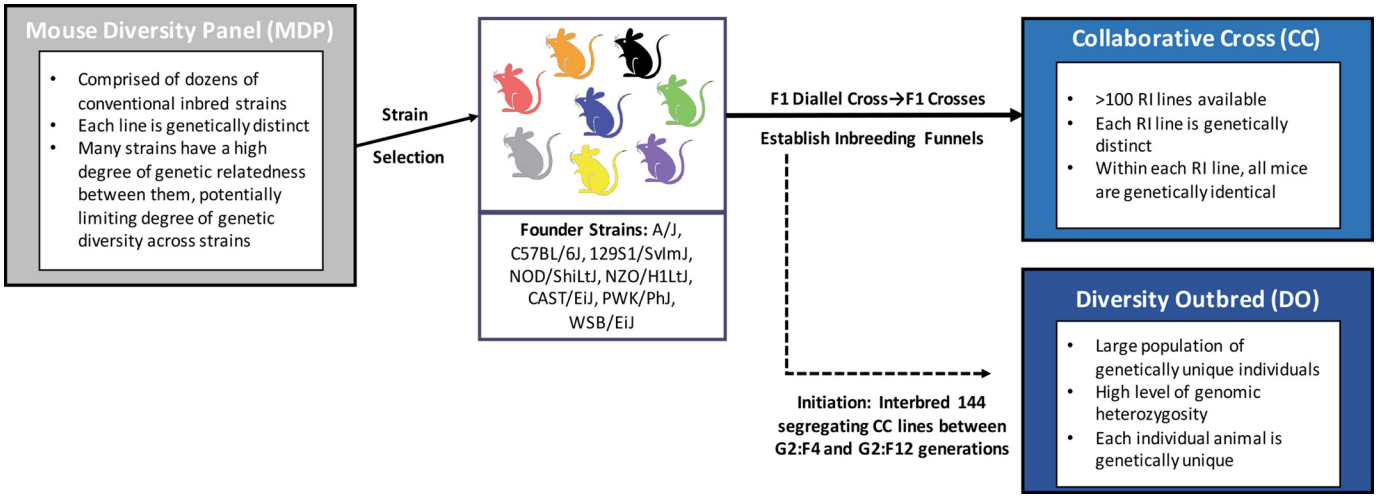

Figure 1. Characteristics of the MDP, DO, and CC RI lines.

mechanistic insights into toxicity outcomes associated with pharmaceutical drugs and chemicals, several of which will be reviewed in later sections.

An advantage of using a mouse population is an ability to exploit the natural genetic variation to identify genetic polymorphisms that drive differential susceptibility to toxicity. An understanding of genetic drivers of toxicity can inform identification of sensitive subpopulations and can also provide insight into the toxicity mode of action. For studies in which genetic analysis is desired (identification of quantitative trait loci; QTL), choice of strains should fall within those for which genetic sequence data are available and with good coverage of polymorphisms throughout the genome. Traditional inbred *M. m. musculus* strains have minimal levels of intrastrain polymorphisms. Thus, it is prudent to select panels that include *M. m. musculus* strains and *M. m. domesticus* strains. The inclusion of *M. m. castaneous* strains in an MDP can be detrimental to a genetic mapping study in that many false positive associations may arise simply due to the genetic divergence of these lines. However, it should be mentioned that there have been tools developed to address the confounding effects of population structure across inbred strains; a popular statistical method for QTL mapping for this population is Efficient Mixed-Model Association (EMMA), available as an R package (Kang et al. 2008).

Although there are pitfalls to using multiple classical inbred lines for genetic mapping studies, there are also some positive aspects to using this population for toxicology. The first is the availability of historical data for many strains (for example, C57BL/6J or Balb/cJ) that may be useful to replicate. Data for many strains may be obtained in the public Mouse Phenome Database (http://phenome.jax.org) (Grubb et al. 2014). In addition, there is the availability of mouse embryonic fibroblast (MEF) lines for many strains, which may be an attractive choice for *in vitro* analyses while MEF and stem cell lines for next-generation mouse populations are still under development (Suzuki et al. 2014). It is important to note that strains that have a similar name (e.g., C57BL/6N and C57BL/6, for which the last letter indicates that these are closely related but not genetically identical substrains) can have genetic sequence differences owing to genetic drift that has occurred where the population is housed (Festing 2010). There is also the potential for genetic contamination, spontaneous mutations, and genetic drift to occur even in an established line if the strain has been maintained over many generations (Wiles and Taft 2010). Therefore, it is important to have consistency when obtaining inbred strains. For these reasons, some vendors now "reset" strains after a defined number of breeding generations using cryopreserved embryos derived from the original stock.

A major consideration for using conventional strains is their availability. Although adult mice of many strains can be purchased in small numbers from several vendors, cost varies widely by strain owing to strain demand, special housing conditions needed for certain strains, and breeding success. Inbred strains are also subject to reduced fecundity and reduction in general health, which has the potential to cause unexpected results in a toxicity study.

Collaborative Cross and Diversity Outbred Mice

Newly developed population-based rodent resources were designed to reflect the genetic diversity of the human population; these resources provide opportunities to investigate population-level responses in a variety of contexts, including disease susceptibility, aging, and adverse responses to chemicals. However, it should be noted that these mouse reference populations were largely intended to be utilized for the identification of genetic risk factors of various pathophysiological states and disease susceptibility—where possible, we direct the reader to tools and resources that are available to facilitate genetic sequence investigations. Nevertheless, these "next generation" mouse reference populations may be an asset for the fields of environmental health science and toxicology in a variety of contexts throughout the risk assessment paradigm, and we have highlighted proof-of-concept experiments in the following sections.

In order to develop next-generation rodent resources, the Complex Trait Consortium spearheaded an effort to develop mouse populations that *a*) encompassed a maximum of genetic diversity available in the *Mus* genus, *b*) had variation randomized throughout the genome to improve the ability to detect the genetic basis of a biological observation, *c*) had a sufficient sample size to power statistical analyses with a lack of minor alleles prevalent in human populations, and *d*) lacked minor (low frequency) alleles (Churchill et al. 2004; Churchill et al. 2012). This effort resulted in two sister populations, the Collaborative Cross (CC) and the Diversity Outbred (DO) (Figure 1). Both CC and DO populations arise from the same eight genetic cofounder strains that were selected to maximize the amount of genetic diversity in the resulting population. Of these founder strains, five represent the *M. m. musculus* subspecies (129S1/SvImJ, A/J, NOD/ShiLtJ, and NZO/H1LtJ), with the remainder of *M. m.*

castaneous (CAST/EiJ), and more recently wild-derived *M. m. musculus* and *M. m. domesticus* subspecific origin (PWK/PhJ and WSB/EiJ). The CC mice consist of recombinant inbred (RI) lines (also called strains) derived from the eight-way cross (CC RI lines); breeding trios of the CC RI lines are available from the colonies housed at the University of North Carolina at Chapel Hill, Chapel Hill, North Carolina (CC-UNC lines) and Tel Aviv University, Tel Aviv, Israel (CC-TAU lines). An advantage of using CC mice for experimental research is the reproducibility of the CC RI lines and public availability of databases on genetic and phenotypic information in each line.

All CC RI lines were independently bred in a scheme that combined the genetic variation present in the eight founder strains into CC RI lines over three generations followed by inbreeding within each breeding funnel to homozygosity (Iraqi et al. 2014) (Collaborative Cross Consortium 2012). CC RI lines are considered complete once they have reached 98% homozygosity and are considered distributable for researchers at 90% homozygosity, which is verified using genetic information obtained via specialized genotyping arrays for this purpose [i.e., the Mouse Universal Genotyping Array (Yang et al. 2009)]. Owing to the availability of genotyping and bioinformatics resources for CC and DO mice, users will have access to an unprecedented level of detail on the genetic sequences of the CC RI lines (Welsh et al. 2012). The CC RI lines have great potential to be utilized for both toxicity analysis and for pharmaceutical efficacy studies involving certain disease states—as more phenotypic information is collated from this population, information regarding spontaneous disease will become more readily available. A positive aspect of the CC RI lines is the reproducibility of members within a line, providing the ability to repeat exposures or test multiple doses in the same genetic context. This also means that if a certain CC RI line is identified as a suitable model for the desired outcome, there is a potential to test next-in-class drugs or chemical isomers in a single selected CC RI strain. The current limitations in availability of animals may affect study timelines.

CC RI lines have been successfully utilized to dissect genetic traits in several contexts. Simulation analysis has proposed that an "idealized minimum" number of animals for a genetic study involving CC RI lines is 128 lines. For a QTL with additive effects, using 128 strains enables detection of a major QTL with an effect size of 0.25 and 90% power (Tsaih et al. 2005). For comparison, the same statistical model indicates that power is reduced to 60% when the CC panel is reduced to 64 strains. Another recent retrospective analysis that included metrics of heritability and the genetic coefficient of variation confirmed that it may be possible to identify a strong QTL that maps to a resolution as narrow as 1 Mb with as few as 100 CC RI lines (Iraqi et al. 2014). It should be noted that the large numbers of mice needed to power a genetics study will, in most cases, far exceed the number of mice needed for typical toxicity testing study.

There are at least two types of genetic studies that have been proposed for CC RI lines. The first type involves genomic mapping of traits measured directly in the standard CC RI lines as described above. A variant of this analysis that has been proposed is called a recombinant inbred intercross or RIX (formally known as a diallel cross). By generating RIX lines, investigators can evaluate parent-of-origin effects, as well as determine whether a gene variant is dominant or recessive, which cannot be detected in inbred parental CCs (it should be noted that these effects may also be evaluated in the DO). An advantage of utilizing RIX lines is that the mice are no longer fully inbred, which can improve the vitality of the animals by introducing hybrid vigor. RIX (like F1 hybrids in general) have lower phenotypic variances than their parental inbreds, which increases phenotyping accuracy. Although the combinatorial effect of a RIX cross increases the number of unique recombinant genomes available (Threadgill et al. 2002; Zou et al. 2005), the increase does not overcome the limitation of having a small panel of RI lines (Tsaih et al. 2005). An advantage of a RIX cross is that the frequency of false positive associations that result from nonsyntenic lineages is reduced in genomic mapping analysis. However, studies that investigated the utility of using RIX lines versus traditional RI panels have concluded that there appears to be little advantage of RIX crosses for genomic mapping analysis (Tsaih et al. 2005).

The UNC Computational Genetics group has developed a suite of tools for viewing and analyzing genetic data from the CC RI lines. One such tool is TreeQA, which is a tree-based association mapping method that can incorporate evolutionary history of the genome into the analysis. Essentially, the algorithm utilizes local phylogenies constructed in genomic regions that exhibit no evidence of historical recombination (Pan et al. 2009). Other packages that have been utilized for the analysis include HAPPY. HBREM (Vered et al. 2014), which employs a logistic regression model to fit covariates. Residuals from the model are then used as the response variable for QTL mapping using linear regression, with the Bayesian random effects model HBREM used to estimate individual haplotype effects (Durrant and Mott 2010).

DO mice are a complementary mouse population model that was derived through the CC development pipeline. DO mice are a heterogeneous stock derived from the same eight founder strains as the CC. Independent lineages (144 in total) were initially selected from the CC breeding colony while the lines were still segregating, and these mice were utilized to seed the DO population. Each DO mouse is genetically unique. DO mice are currently maintained as a randomized breeding colony of 175 breeding pairs. DO mice are robust and breed efficiently, averaging seven pups (± 2.4 SD) in first litters (Churchill et al. 2012). Each DO mouse harbors a high level of heterozygosity, with the population at large providing a vast array of allelic combinations. This level of heterozygosity is maintained by randomly selecting a male and female from each first litter and assigning to a new breeding pair to generate the next generation. Such a mating scheme doubles the effective population size, minimizes genetic drift, and minimizes selection on the allele frequencies within the population (Rockman and Kruglyak 2008).

The likelihood of severe morbidity and mortality may be lower in the DO as compared with CC or MDP mice, owing to hybrid vigor associated with a high level of heterozygosity. One example of this phenomenon occurred in the MDP investigation of isoniazid-induced hepatic steatosis. In that study, drug-treated members of two inbred strains (P/J and WSB/EiJ) had to be excluded from subsequent analyses owing to mortality that was not associated with liver injury (Church et al. 2014). In studies of DO mice using the same dose level and longer exposures to isoniazid, no mortality has been observed (A.H. Harrill, unpublished data, 2017). For these reasons, DO mice may offer advantages for toxicology studies in which hazard identification is the main goal, particularly in cases where toxicity mode of action is not known. Because the DO mice breed well, with high fecundity, and are bred in three to four large breeding waves per year, it is more straightforward to obtain a large cohort of similarly aged DO mice as compared with CC RI lines.

A common question is whether researchers should first screen a chemical in members of the eight genetic founder strains of the CC and DO to assess study feasibility. In cases where some data exist in one of the founders already (for example, C57BL/6J), it may be reasonable to assess a dose response across the founders to guide dose selection for an expanded population. However, use of the founder strains to inform target organs or dose selection in a

CC or DO study may not be productive. In our studies (A.H. Harrill, unpublished data, 2017), we have observed a lack of toxicity in the founders for drugs and doses where a significant hepatic or renal toxicity response is observed in the DO. In addition, it has been observed by others that the phenotypic variation in the CC (and DO) quite often exceeds that of the founder strains (Philip et al. 2011). An alternative, and perhaps more straightforward strategy, is to conduct a dose range finding study in a modestly sized cohort of DO mice (with the cohort size based on appropriate power calculations). As more studies are conducted using the DO and CC RI lines and data are deposited in online public repositories soon, power calculations will become more straightforward.

Going beyond classical toxicity studies, CC and DO mice are being increasingly utilized for a variety of genome-wide association study (GWA or GWAS), in which a genome-wide set of genetic variants is analyzed in different individuals to identify regions of the genome that contain sequence variation that significantly influences the experimental outcome (e.g., degree of toxicity as measured by a biomarker). DO mice typically require greater numbers of animals for a GWA study as compared with CC mice. The reason is that DO mice have a high level of heterozygosity, whereas CC mice are largely homozygous at every locus. A major consideration for the sample size is that the magnitude of the effect of a gene variant is often not known *a priori*. Therefore, a reasonable experimental strategy could be to start with fewer numbers of DO animals, analyze the GWAS data, and then add more to the study if the necessary power was not achieved. This strategy is more amenable to DO mice now, although it is expected that more CC RI lines will become available in the future to bolster experimentation with CC RI lines. For DO mice, simulated power analyses indicate that as few as 200 mice can be utilized to detect QTL regions with large effects. In contrast, loci that account for $<5\%$ of trait variance may require up to 1,000 mice (Gatti et al. 2014b). However, it is unlikely that loci with very minor effects will have utility as a genetic marker for toxicity susceptibility, and achieving power to detect such a minor effect may be unproductive with regards to desired study outcomes. On the other hand, for projects where it is desirable to explain as much of the trait variance as possible (e.g., for a neurobehavioral outcome), larger numbers of animals may be preferable. In routine studies to identify toxicogenetic markers for susceptibility to xenobiotic toxicity, a rule of thumb is to start with 400 DO mice. Power simulations indicate that with 400 mice, QTL can be detected (at $\alpha = 0.05$) that account for 10% of the phenotypic variance with 80% power. Based on existing data from DO mice at generations 7 and 8 of outbreeding, 400 mice provide a median recombination block width of 0.33 Mb (Gatti et al. 2014b). This resolution is fine enough to map loci down to a handful of genes. A caveat is that, in practice, the local linkage disequilibrium structure will also influence the QTL width.

The currently used software for GWA analysis in DO mice is a Bioconductor package called DOQTL, which can be utilized in R (Gatti et al. 2014a; Gatti et al. 2014b). DOQTL provides a suite of tools for processing DO genotype data, reconstructing individual genomes by inferring sequences using haplotypes from the founder strains, and performing QTL analysis. In addition, the package allows for assessment of founder haplotype effects on the QTL interval.

Use of Mouse Populations in a Risk Assessment Framework

Although many of the studies published using the CC or DO mice thus far have focused on genetic analyses, there are many study types that can be performed using these populations to inform the risk assessment process. In the following sections, we provide recommendations for the utility of these populations at various steps in the process and provide examples of how researchers have applied these models for illustrative purposes. A summary of these recommendations is provided in Figure 2.

Exposure Assessment

Exposure assessment describes how and to what extent humans come into contact with hazards; the principles, concepts, and methods have been elucidated by regulatory agencies, such as the U.S. EPA (1992). The internal dose of a chemical is a key component of exposure; internal dose is the availability of an amount of chemical to biologically significant sites within the body (National Academies of Sciences and Medicine 2012, 2017). An emerging idea is to utilize mouse population-based models to better estimate internal dose and to better understand the relationship between the internal dose and the applied dose using data on bioavailability and pharmacokinetics. It is rare that risk assessors have human toxicokinetic data available to characterize interindividual variability, and therefore default assumptions must be applied. Mouse population resources offer risk assessors an opportunity to quantitatively address interindividual variability in toxicokinetics of chemicals.

The studies demonstrating the use of an MDP to facilitate population physiologically based pharmacokinetic (PBPK) modeling are few, but encouraging. A recent study by Chiu and coauthors that examined metabolism of trichloroethylene (TCE) in 16 inbred mouse strains (and an F1 hybrid) found between a 2- and 10-fold variability in metabolic flux through Phase I and II metabolic pathways (Chiu et al. 2014). Significantly, the population variability estimates attained from the mouse strains were equal to population variability estimates derived previously from human toxicokinetic studies (Chiu et al. 2009). Genetic differences between strains determined the extent to which TCE-metabolism genes were induced following TCE exposure (Bradford et al. 2011).

Although few studies have fully examined toxicokinetic differences in the DO and CC RI lines, data are emerging that will provide key insights to guide future mouse population-based exposure assessments. A recent investigation characterized common metabolic biotransformation pathways in livers derived from 29 CC RI lines (Nachshon et al. 2016). A key finding of the study was that associations between genetic sequence variation and hepatic drug disposition enzymes were related to alternative spicing of the encoding genes. Similarly, variation in glutathione-*S*-transferase genes that play a protective role against oxidative stress have been recently shown to vary widely across mouse RI lines and to have variable expression across tissue types (Lu et al. 2016). As more data become available that catalog metabolic differences between strains and among the DO, rationally designed studies to enable data-driven risk assessments using population data will become more tractable.

Hazard Identification

Hazard identification is a key part of the risk assessment framework during which both toxicokinetic and toxicodynamic studies are utilized to determine whether a given exposure increases the incidence of adverse effects. Key to this process is determination of whether the adverse effects is likely to occur in humans. Mouse population–based studies provide a method by which adverse events that only occur in genetically sensitive individuals may be detected and incorporated into the analysis. In contrast, single-strain studies have the potential to miss adverse outcomes that occur in humans. Potential opportunities for this model lie in projecting the estimated extent of occurrence of an adverse effect, defining segments of the population that may be particularly susceptible (i.e., through termination of genetic predisposition as a

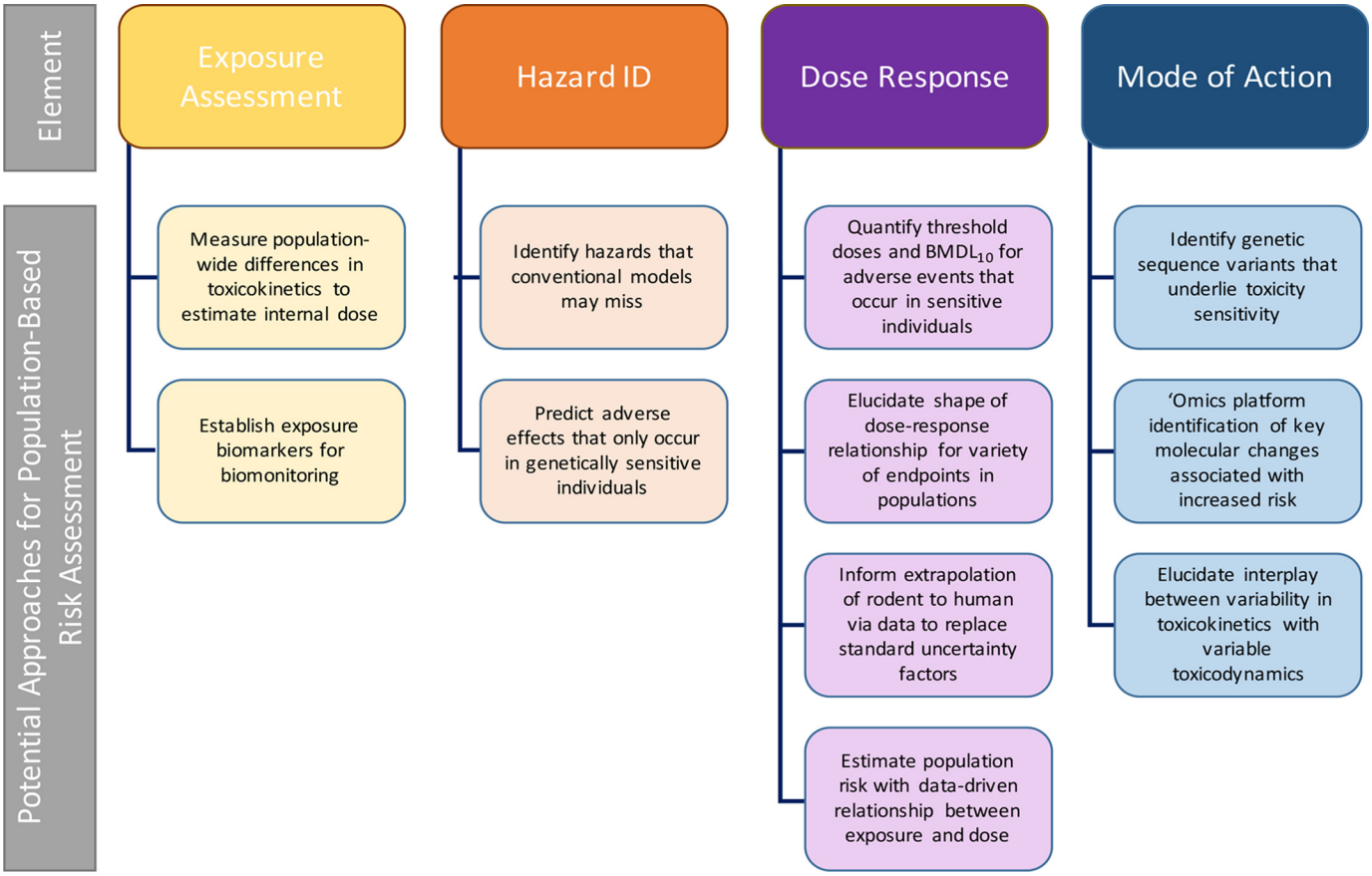

Figure 2. Potential uses of mouse populations through the risk assessment paradigm.

risk descriptor), and determining the distribution of risk among population subgroups. Mouse population models also have the potential to define the shape of a dose–response curve (linear, nonlinear), the arithmetic mean risk, and subsequently a probalistic estimate of the extent of effects.

An example of this concept is a recent investigation into the ketolide antibiotic PF-04287881, which was abandoned from pharmaceutical development following observation of liver biomarker elevations in clinical trials that were not predicted by classical rodent stocks used in preclinical screens (Mosedale et al. 2014). In that study, an MDP approach was used and a subset of susceptible mouse strains sustained liver injury in response to the drug; mouse strains that experienced liver injury were subsequently found to exhibit phospholipidosis in Kupffer cells. This finding was not entirely surprising given that PF-04287881 is a cationic amphiphilic drug (CAD) and that phosopholipidosis has been associated with previously developed CADs, including antiarrhythmics, antidepressants, and other antibiotics (Halliwell 1997). Importantly, use of multiple mouse strains for hazard identification yielded the clinically relevant toxicity; whereas conventional rodent testing did not.

In the case of PF-04287881, the differences in susceptibility between strains could then be utilized to study the mode of action of liver injury. Once susceptible and resistant strains were identified using biochemical and histopathological criteria, a subset of four strains was utilized for global transcriptomic analysis of liver tissue. Pathway analysis of transcripts that were altered by the drug and that were uniquely affected in susceptible strains indicated that PF-04287881-induced phospholipidosis was mediated by alterations in phospholipid metabolism and lysosomal function. Furthermore, the liver injury caused by PF-04287881 exposure was linked to changes in expression of genes involved in protein degradation, potentially leading to accumulation of oxidized proteins (Mosedale et al. 2014).

In a similar study, an MDP approach was utilized to determine whether genetically sensitive mouse strains could have predicted clinically significant kidney injury that occurred in an expanded Phase 1 clinical trial of a novel drug for treatment of African trypanosomiasis, DB289 (Harrill et al. 2012). As in the previous example, severe renal injury caused by DB289 in clinical trials was not indicated during conventional toxicity testing in rodents or nonhuman primates. The clinical trial data indicated a potential link between genetics and susceptibility to DB289-induced renal injury because renal toxicity was only apparent in study subjects enrolled in South Africa, and was absent from subjects enrolled in trials in the countries of Angola, the Democratic Republic of the Congo, and Sudan. Within the mouse panel, several strains were identified that experienced renal injury caused by DB289 administration, providing another example of a case where a mouse population model could identify human relevant toxicities that were absent from conventional screens.

Additional recent proof of principle studies has further demonstrated the capability of these models to characterize susceptibility to chemically induced adverse effects, lending credibility for their use in toxicology research and risk assessment. For example, it was recently shown that there is up to 3-fold variation across CC RI strains in the activity of mitochondrial respiratory complexes I-IV, providing opportunities to identify hazards associated with agents that disrupt cellular respiration (Hartman et al. 2017).

Dose Response

There is growing interest in utilizing DO mice to set exposure thresholds for chemicals that may be occupational hazards. A

landmark study that employed the DO for this purpose investigated whether current guidelines set for benzene exposures in an occupational setting were adequately protecting both sensitive and resistant workers (French et al. 2015). Typical experiments to identify the "benchmark dose (BMD)" or point-of-departure for adverse effects are conducted in genetically homogeneous B6C3F1/J mice. In this DO study, a dose-dependent increase in benzene-induced chromosomal damage was observed, with a wide degree of susceptibility reported across the population of DO mice. Furthermore, the benchmark dose lower bound 10% ($BMDL_{10}$) was an order of magnitude lower in the DO mice as compared with B6C3F1/J mice, and the DO-based estimate of 0.205 ppm benzene was consistent with human exposure data (Lan et al. 2004). These data suggest that that the increased genetic diversity in the DO could be exploited to identify a more accurate concentration threshold that could protect more workers from the adverse effects of benzene exposure.

Mode of Action

A mouse population–based approach may also be employed to inform the mode of action for toxicity. A recent demonstration of this application is a study that utilized a panel of inbred strains to investigate the molecular mechanisms of genetic sensitivity to isoniazid-induced steatosis (Church et al. 2014). The study was conducted as a consortia effort by members of the Health and Environmental Sciences Institute (HESI)'s Application of Genomics to Risk Assessment Technical Committee. In that study, a combined transcriptomic, metabolomic, and pharmacogenomic analysis was utilized to provide evidence for a novel hypothesis that isoniazid increases the capacity for formation of lipid droplets while concurrently reducing the capacity for exporting stored fat from the hepatocytes in sensitive strains. A key aspect of this approach is that a subset of sensitive and resistant strains may be selected for downstream analysis after an initial screen of a larger strain panel. By selecting strains with certain characteristics, it is possible to reduce costs and complexity of targeted experiments to elucidate toxicity mechanisms.

Mouse population models may additionally be utilized to identify quantitative biomarkers that are associated with toxicity sensitivity. An example of this approach is a recent transcriptomic study that was conducted using a panel of inbred strains (Harrill et al. 2009a). In that study, strains were identified as either sensitive or resistant to a high oral dose of acetaminophen. Global mRNA transcript expression was measured in livers extracted from all the strains and the data were analyzed such that strain (genetic background), treatment (acetaminophen or vehicle), and liver injury score (% necrosis) were used as factors in an ANCOVA model (analysis of covariance). This analysis allowed for discernment of transcripts with expression that changed *a*) with treatment, but not with strain; *b*) with strain, but not with treatment; and *c*) with treatment and strain, and that varied with the amount of hepatocellular necrosis. The 26 transcripts in the latter group represented genes involved in cell death and proliferation. This same experimental paradigm could be extended to metabolomic or proteomic analyses to identify accessible biomarkers that indicate toxicity mode of action in genetically sensitive individuals.

An added value of using mouse population models for toxicology is the opportunity to determine the genetic basis for susceptibility to an adverse effect. An understanding of the genetic basis can be informative for informing the toxicity mode of action in susceptible individuals, and for informing development of fit-for-purpose toxicity screens that exploit knowledge of the genetic basis of an adverse effect. Whole-genome efforts toward genetic dissection of toxicity traits is aimed at determining genomic locations (quantitative trait loci; QTL) that contain genetic variation that influences the toxicity response of interest. Discovering quantitative trait genes (QTGs) in mouse reference populations that affect toxicity susceptibility have the potential to be translated into humans, especially where there is high sequence homology (or reasonable orthology of the resultant protein) between mouse and human species for the QTG of interest.

Extensive research in recent years, including the Human Genome and HapMap projects, has led to a wealth of information on genetic polymorphisms in human populations (Manolio et al. 2008). It is reasonable to expect that there are many polymorphisms that contribute to adverse xenobiotic responses (or disease) owing to the observation that base pair sequence variation among individuals averages 1 in 500–1,000 base pairs (Venter et al. 2001). It is now possible to identify single nucleotide polymorphisms (SNPs) that can serve as biomarkers for identifying genetically sensitive individuals or subpopulations of individuals. Predictive genetic tests may have value in risk prediction and may yield insights into the mode of action of toxicity for xenobiotic agents. An example of this concept is the genetic testing available to patients with HIV who are prescribed the drug abacavir, in which screening for major histocompatibility complex, class I, B (HLA-B) *5701 substantially reduces the risk of hypersensitivity reactions (Mallal et al. 2008).

There are several recent examples for which mouse reference populations have been utilized for identification of genetic risk factors for toxicity outcomes. An exemplar case is a study that investigated behavioral, neurochemical, and transcriptomic responses to chronic exposure to fluoxetine. In this MDP study, it was observed that genetic background greatly influenced the therapeutic response, with 5/30 strains identified as negative responders, 13/30 strains identified as positive responders to the medication, and the remainder of the strains identified as nonresponders (Benton et al. 2012). Genetic association was found with the gene encoding cellular proliferation/adhesion molecule (*Cadm1*), which may indicate a potential role for neuro/gliogenesis in depression. Similarly, the MDP reference population was utilized to identify genetic risk factors for liver injury caused by acetaminophen overdose. A gene candidate for acetaminophen-induced hepatotoxicity, *Cd44*, was identified by GWA in mice. A SNP within this gene was subsequently found to have a clinical association with both asymptomatic elevations in liver function tests as well as acute liver failure due to acetaminophen overdose in susceptible patients (Harrill et al 2009b). Such an approach has implications for mouse-to-human translational pharmacogenetics approaches to precision prescribing of medicines. In addition, understanding the genetic basis of toxicity, and knowledge of human gene frequencies in a geographic region, may influence risk assessment for chemicals in the future.

Mouse embryonic fibroblasts (MEFs) that have been derived from MDP strains may add utility to high-throughput investigation of drug-induced toxicity risks. A recent study reported the screening of 65 compounds across 32 strains (Suzuki et al. 2014). A success of the study was that a QTG was identified that was associated with rotenone toxicity. This gene, *Cybb*, was subsequently validated experimentally both *in vitro* and *in vivo*, providing a promising test case for utilizing cell-based screens for pharmacogenetic identification of gene variants that confer toxicity risk (Suzuki et al. 2014).

There are some drawbacks to using an MDP for use in mode of action studies that aim to identify a genetic basis for an adverse response. The genetic variation within panels of inbred mouse strains, although greater than that of standard recombinant inbred (RI) lines, can be somewhat limited given that most classical inbred strains are derived from *M. m. domesticus*. Genetic variation among classical inbred strains is both limited and unevenly

distributed across the genome (Yang et al. 2007; Yang et al. 2011). The CC RI lines and DO stock were designed to incorporate large genetic variation (Churchill et al. 2004). Both the CC and DO mice have the potential for genomic mapping resolution down to 1-Mb genomic intervals, which affords higher resolution genomic mapping than what is possible with an MDP. At each successive generation, DO mice accumulate approximately 25 recombination breakpoints. By generation 10, DO mice harbored approximately 350 breakpoints, a greater number than the typical F2 mouse, which harbors approximately 28 breakpoints. This large number of breakpoints drives tight localization of QTL peaks, whereas a very large number of sequence variants drives increased sensitivity to detect QTLs in the DO mice.

In the DO study on differential susceptibility to benzene-induced mutagenesis, genomic mapping was performed to identify QTGs that were associated with benzene toxicity in DO mice, as measured by the micronuclei frequency of reticulocytes from peripheral blood and bone marrow. The investigators identified a highly significant peak (LOD > 20) on chromosome 10 that contained several genes. Analysis of the effects of founder effect haplotypes within the QTL interval enabled determination that DO mice who had inherited the CAST/EiJ founder haplotype were less sensitive to benzene versus DO mice without the haplotype. *Sult3a1* was identified as a candidate gene in the region because it contained alleles that are unique to the CAST/EiJ haplotype. Interestingly, examination of mRNA expression patterns of this gene in the eight founder strains led to the discovery that CAST/EiJ mice have a greater expression of *Sult3a1* and this founder strain harbors a copy number expansion of the gene. Thus, DO mice that harbor the CAST/EiJ haplotype likely have a greater capacity to detoxify benzene, providing a foundation for resistance to benzene-induced chromosomal damage. The workflow utilized in the benzene study can provide a template for gaining mechanistic insight into adverse outcomes associated with drugs or chemical exposures.

In another recently publish DO toxicity study, genetic risk factors for susceptibility to green tea extract–induced liver injury were explored (Church et al. 2015). The goal of the study was to better understand why certain consumers were susceptible to liver injury to herbal supplements that contained green tea extract, which was particularly intriguing because there was no apparent dose relationship in the affected individuals (idiosyncratic effects) (Navarro et al. 2013). In a large population of DO mice exposed to the major constituent of green tea extract (epigallocatechin gallate), it was found that a small fraction of the animals (16%) sustained a high degree of hepatocellular injury analogous to the severe human clinical cases. Toxicity testing illustrated the difference in susceptibility across populations with 35% of the animals tested being completely resistant to the toxicity (i.e., no evidence of adverse liver histopathology). Genetic analysis revealed that sequence variation within a region on chromosome 4 was associated with the toxicity in mice. Sequence analysis in human clinical cases of liver injury arising from green tea extract–containing supplements corroborated the findings with significant enrichment in the cases versus controls of variants in three genes; one of which—*Mitofusin 2* (*MFN2*)—emerged as a particularly interesting candidate risk factor owing to its role in mitochondrial maintenance and autophagy as well as promotion of cellular death under stress conditions (Papanicolaou et al. 2011). Epigallogathechin gallate was subsequently shown to inhibit mitochondrial respiratory complexes under stress conditions (Weng et al. 2014), suggesting that an interplay between genetic risk factors and the environment may play a role in clinically important liver injury due to this compound.

High-Throughput Population Variability Measurements in Cell Cultures

Although low-throughput dose–response and classical toxicological analyses to understand population variability are tractable *in vivo*, the high cost of *in vivo* population studies has generated significant interest in *in vitro* models for toxicity screening. Potential applications include: quantitative dose–response modeling of adverse effects, screening libraries of potentially hazardous compounds for toxicity and functional responses, establishing molecular signatures of exposure, QTL/eQTL (expression QTL) mapping to find genetic variants underlying differential susceptibility to various environmental exposures, identifying susceptible mouse strains for targeted *in vivo* studies, validating environmental response networks (using knockdowns), and validating genetic associations by genome editing (such as CRISPR-Cas9).

Because many of these resources are still in development, published studies demonstrating proof-of-concept are lacking. We reviewed in previous sections recent successes in genomic mapping using mouse embryonic fibroblast cells derived from an MDP. There are also parallel efforts ongoing to develop embryonic stem cells from DO and from CC mice. Predictive Biology, Inc. (Carlsbad, CA) currently offers testing in the following cell types derived from DO male and female mice: embryonic stem cells, cardiomyocytes, and neural progenitor cells. The DO ES lines have been shown to be euploid and stable (T. Choi, written communication, May 2016). Cell-based screens using mouse population cell systems can be used to perform high-throughput genome-wide analyses of chemical-induced toxicity that can subsequently be functionally validated using human induced pluripotent stem cell (iPS)–derived cells and gene knockdown/knockout experiments.

Mapping complex traits with DO ES lines offers distinct advantages to human induced pluripotent stem (iPS) cell lines. Power calculations have shown that a panel of at least 10,000 human iPS cell lines would be needed to match the mapping power of 400 DO ES cell lines for GWA studies. This is a consequence of the very low allele frequency of many functional variants in the human genome. Because of the rapid fixation of rare alleles occurring during brother–sister mating during the generation of the eight inbred founder genomes of the DO, nonsynonymous SNPs (nsSNPs) in the DO have minor allele frequencies of 12.5–50%. This is in sharp contrast to over 96% of nonsynonymous coding SNPs in human populations having allele frequencies of 0.5% or less, with more than half of these found only once in 2,500 human genomes.

Mouse Population Screens Yield Fit-for-Purpose Disease Models That May Be Used to Define Sensitive Subpopulations

An emerging concept has been to investigate toxicity in the context of co-morbidities, such as obesity or diabetes, but there are opportunities to investigate adverse outcomes in a variety of disease states if the "right" animal model could be identified. Panels of CC RI lines have been utilized in several efforts to identify models of disease, particularly in cases in which animal models are either lacking or have been reported as ineffective using common rodent strains. For example, research of therapies for Ebola virus infection using the mouse adapted strain of Ebola virus (MA-EBOV) was historically restricted to macaques, guinea pigs, and Syrian hamsters, owing to a failure of common mouse strains to reproduce the hemorrhagic hallmarks of the human disease. In a landmark study utilizing 47 recombinant inbred intercross lines of CC mice (CC-RIX), researchers found that genetic background played an important role in the pathogenesis of

Ebola infection, with a phenotypic range across CC-RIX hybrids ranging from complete resistance to severe pathology that was consistent with Ebola hemorrhagic fever and lethality (Rasmussen et al. 2014). In addition to these observations, it was observed that select CC-RIX hybrids experienced lethality without symptoms of Ebola hemorrhagic fever. Thus, a screening strategy to identify CC RI lines or CC-RIX hybrids that exhibit pathology consistent with human disease may advance therapeutic development.

In addition, spontaneous disease observed in CC RI lines may provide unique opportunities for identifying mouse models of human medical conditions. One such possibility is the study of inflammatory bowel disease (IBD), an immune-mediated condition that is modulated by aberrant responses to intestinal microflora under certain host genetic and environmental contexts. Although rodent models of IBD had existed, prior models were reliant upon interventions to induce the disease such as a chemical induction or introduction of an infectious agent. A CC line that has been identified as a model for IBD was discovered fortuitously when it was observed that there was a high frequency of spontaneous rectal prolapse in the CC011/Unc line (Rogala et al. 2014). Following observance of the prolapse, animals generally maintained a good body condition; however, the prolapsed tissue became necrotic or ulcerated, resulting in deteriorating conditions that necessitated euthanasia for humane reasons in the affected animals. Affected animals in the line were found to have hallmark features of colitis by histological assessment of gastrointestinal tissues, and no coincident pathogenic infection was found.

The utility of using DO mice to model human disease and population-level responses has been demonstrated in recent manuscripts as well. As with CC mice, DO mice have been employed as a tool to study a variety of human conditions, including pain sensitivity (Recla et al. 2014), development of atherosclerosis (Smallwood et al. 2014), susceptibility to *Mycobacterium tuberculosis* infection (Harrison et al. 2014), and neurobehavioral traits (Logan et al. 2013), including addiction to drugs of abuse (Dickson et al. 2015).

As new mouse models for complex human disease outcomes become available and characterized through these new population-based rodent resources, many possibilities for exploring the understanding of gene–environment interactions and genetic susceptibility to exposures could be undertaken with controlled exposure experiments using these animals.

Considerations for Study Design

It is important to note that the populations under discussion may all be suitable for standard toxicology screens; however, as discussed extensively in the NIEHS workshop, it may be advisable to reserve use of this population for cases in which assessment of population variability is both desirable and tractable (based on power/sample size calculations). Practical considerations, such as availability of strains, should be considered when selecting a mouse reference population for study.

The phenotype (or experimental outcome) measured is an important consideration in the design of studies involving mouse populations. Because mice of various genetic contexts will likely exhibit different outcomes, it is important to choose a phenotype for which sampling and measurement error can be minimized. The more precise the quantitatively measured outcome is, the less noise there will be in the dataset, and the better the estimate of variance will be. This is especially critical in cases where the intent is to use quantitative phenotype information as a starting input for the GWA models, but is less of a concern for studies aimed at identifying chemical hazards. Examples of ideally suited phenotypes for the purposes of genetic mapping can include, but are not limited to: quantitative pathology scores, organ weights, or quantitative tissue markers. Special consideration should be given to utilizing blood-based leakage biomarkers as there may be differences in transport and clearance rates among genetically diverse animals that could affect endpoint measures. With that caveat aside, circulating biomarkers have been used successfully to gain insights into toxicity outcomes within mouse reference populations.

Although much attention has been focused on the utilization of population-based rodent models for toxicity testing and to identify genetic causes of susceptibility to toxicity, it is becoming increasingly apparent that epigenomic changes play a large role in toxicant responses. Interstrain susceptibility to genotoxicity for well-established toxicants is sometimes likely due to strain-specific epigenetic events in response to the exposure. Therefore, additional challenges will be to assimilate epigenomic data with genetic and other omics data for these population-based rodent models and better predict risks as modulated by epigenetic changes (Koturbash et al. 2011).

Recent approaches have enabled meta-analysis across rodent population studies to identify environmentally specific gene effects. A unique meta-gene by environment (G × E) approach by Kang et al. was recently conducted across 17 mouse studies (totaling 4,965 animals) for detection of significant loci that influence high-density lipoprotein cholesterol levels (Kang et al. 2014). The meta-analysis framework allowed disparate mouse studies with varying environmental conditions to be analyzed jointly in a model that treated gene–environment interactions as random effects. The potential utility of such a model is to combine many smaller studies with different environmental exposures for the purposes of obtaining significantly higher power and improved genetic mapping resolution. This meta-G × E strategy may provide a useful approach for identifying gene by environment interactions that underlie the architecture of complex traits and is particularly powerful when applied to the analysis of studies using mice of varying genetic backgrounds that have been conducted in different environments.

Beyond the scope of this article, it should be noted that another variable not currently often considered that can have profound impacts on toxicity testing are common environmental impacts such as diet. Most toxicity testing is performed in animals maintained on standardized lab chows that do not recapitulate the typical diet of Western countries. Environmental factors should be well controlled and described to minimize undesired gene–environment effects.

Rat Resources

Significant progress has been made in recent years toward using the rat as a species for population-based modeling as the rat has long been established as a useful model for certain phenotypes relevant to human physiology and behavior. Many classical toxicology studies have been performed in rat models owing to their relatively large size that increases the amount of tissue and biofluids that can be harvested for the purposes of toxicologic pathology. Several rat models are available for assessment of genetic variability, including, but not limited to *a*) lines bred selectively for various traits, such as alcohol preference (McBride et al. 2014); *b*) inbred strains and recombinant inbred lines, such as the HxB/BxH RI lines that are derived from SHR and BN-Lx rats (STAR Consortium et al. 2008; Vanderlinden et al. 2014); *c*) heterogeneous outbred stocks (Solberg Woods 2014); and *d*) genetically modified rats (Li et al. 2013). Work is currently underway to develop and characterize a Hybrid Rat Diversity Panel (HRDP); a project that will result in a panel of 30 recombinant inbred lines derived from an original set of nine founder rat strains (B. Tabakoff, written communication, October

2016). Population-level investigations can take advantage of the HRDP and HxB/BxH strains in combination to broaden the genetic space that can be queried.

Although detailed genetic information for the rat has lagged behind that of mouse and human species, there are a growing number of data rich bioinformatics resources that include the Rat Genome Database and transcriptome browser PhenoGen (Bhave et al. 2007; Shimoyama et al. 2015). Recent studies have utilized various rat population resources for identification of gene variants associated with metabolic syndrome (Gauguier et al. 1996) and metabonomic traits in the context of diabetes (Dumas et al. 2007). These resources provide a platform toward using rat models for assessing genetic susceptibility to environmental exposures.

Final Considerations

The next wave of population-based risk assessment is the ability to predict human-relevant chemical hazards and to account for their differential sensitivity to xenobiotic agents in dose–response modeling and other assessments. Furthermore, regulatory agencies, industry, and academics must establish clear guidelines for the integration of population variability into toxicity testing guidances.

A future direction of population-based genetic research is the ability to tailor risk management for uniquely sensitive subpopulations. Translational aspects in this area will be greatly facilitated by additional proof-of-concept examples using pharmaceutical agents for which human data are available. Although there are promising data that show that GWA can predict human toxicity responses to xenobiotics across diverse populations, further examples demonstrating that this method can accurately identify human-relevant risk factors is necessary. Studies in this arena are ongoing and we can expect additional published examples soon.

It will take a period of research and development to fully characterize background pathology and biomarker reference ranges for each mouse population that can serve as a basis of comparison across toxicity studies. The NTP has a manuscript in preparation describing clinical pathology reference ranges for the DO and data for the CC are emerging. A potential additional use of this population for toxicology applications is to test candidate toxicity biofluid-based biomarkers. Population models allow for testing of biomarker performance in a population space to determine whether a biomarker has improved specificity and sensitivity for detecting organ injury over conventional gold-standard biomarkers.

Significant consideration will need to be given to how to incorporate mouse population-level data into current paradigms. For example, it will be important to appropriately account for variability within populations within benchmark dose assessments. An open question is how to account for the variability without potentially overestimating risk to human populations; however, it is expected that methods tailored toward population-based assessments (such as those used in epidemiological studies and population studies of pharmacokinetics and pharmacodynamics) may be appropriated for this purpose. This is a subject that is an area of active investigation and which will require significant dialog between risk assessors, toxicologists, and basic researchers with experience in these mouse populations.

For the potential of mouse population models for risk assessment applications to be fully realized, greater emphasis should be given to collaboration across laboratories and disciplines. Team research within centers and consortia will greatly facilitate routine adoption of these models. Together, mouse reference populations have the potential to provide a framework for integrating population variability into the study of toxicity outcomes. These populations provide a foundation for the integration of molecular, morphological, pathological, and physiochemical data toward a holistic understanding of the genetic basis of toxicity susceptibility.

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References

Benton CS, Miller BH, Skwerer S, Suzuki O, Schultz LE, Cameron MD, et al. 2012. Evaluating genetic markers and neurobiochemical analytes for fluoxetine response using a panel of mouse inbred strains. Psychopharmacology (Berl) 221(2):297–315, PMID: 22113448, https://doi.org/10.1007/s00213-011-2574-z.

Bhave SV, Hornbaker C, Phang TL, Saba L, Lapadat R, Kechris K, et al. 2007. The PhenoGen informatics website: tools for analyses of complex traits. BMC Genet 8:59, PMID: 17760997, https://doi.org/10.1186/1471-2156-8-59.

Bradford BU, Lock EF, Kosyk O, Kim S, Uehara T, Harbourt D, et al. 2011. Interstrain differences in the liver effects of trichloroethylene in a multistrain panel of inbred mice. Toxicol Sci 120(1):206–217, https://doi.org/10.1093/toxsci/kfq362.

Chiu WA, Campbell JL Jr, Clewell HJ III, Zhou YH, Wright FA, Guyton KZ, et al. 2014. Physiologically based pharmacokinetic (PBPK) modeling of interstrain variability in trichloroethylene metabolism in the mouse. Environ Health Perspect 122(5):456–463, PMID: 24518055, https://doi.org/10.1289/ehp.1307623.

Chiu WA, Okino MS, Evans MV. 2009. Characterizing uncertainty and population variability in the toxicokinetics of trichloroethylene and metabolites in mice, rats, and humans using an updated database, physiologically based pharmacokinetic (PBPK) model, and Bayesian approach. Toxicol Appl Pharmacol 241(1):36–60, PMID: 19660485, https://doi.org/10.1016/j.taap.2009.07.032.

Church RJ, Gatti DM, Urban TJ, Long N, Yang X, Shi Q, et al. 2015. Sensitivity to hepatotoxicity due to epigallocatechin gallate is affected by genetic background in diversity outbred mice. Food Chem Toxicol 76:19–26, PMID: 25446466, https://doi.org/10.1016/j.fct.2014.11.008.

Church RJ, Wu H, Mosedale M, Sumner SJ, Pathmasiri W, Kurtz CL, et al. 2014. A systems biology approach utilizing a mouse diversity panel identifies genetic differences influencing isoniazid-induced microvesicular steatosis. Toxicol Sci 140(2):481–492, PMID: 24848797, https://doi.org/10.1093/toxsci/kfu094.

Churchill GA, Airey DC, Allayee H, Angel JM, Attie AD, Beatty J, et al. 2004. The Collaborative Cross, a community resource for the genetic analysis of complex traits. Nat Genet 36(11):1133–1137, PMID: 15514660, https://doi.org/10.1038/ng1104-1133.

Churchill GA, Gatti DM, Munger SC, Svenson KL. 2012. The diversity outbred mouse population. Mamm Genome 23(9–10):713–718, PMID: 22892839, https://doi.org/10.1007/s00335-012-9414-2.

Collaborative Cross Consortium. 2012. The genome architecture of the Collaborative Cross mouse genetic reference population. Genetics 190(2):389–401, PMID: 22345608, https://doi.org/10.1534/genetics.111.132639.

Collins SL, Carr DF, Pirmohamed M. 2016. Advances in the pharmacogenomics of adverse drug reactions. Drug Saf 39(1):15–27, PMID: 26650062, https://doi.org/10.1007/s40264-015-0367-8.

Cote I, Andersen ME, Ankley GT, Barone S, Birnbaum LS, Boekelheide K, et al. 2016. The Next Generation of Risk Assessment multi-year study—highlights of findings, applications to risk assessment, and future directions. Environ Health Perspect 124(11):1671–1682, PMID: 27091369, https://doi.org/10.1289/EHP233.

Dickson PE, Ndukum J, Wilcox T, Clark J, Roy B, Zhang L, et al. 2015. Association of novelty-related behaviors and intravenous cocaine self-administration in Diversity Outbred mice. Psychopharmacology (Berl) 232(6):1011–1024, PMID: 25238945, https://doi.org/10.1007/s00213-014-3737-5.

Dumas ME, Wilder SP, Bihoreau MT, Barton RH, Fearnside JF, Argoud K, et al. 2007. Direct quantitative trait locus mapping of mammalian metabolic phenotypes in diabetic and normoglycemic rat models. Nat Genet 39(5):666–672, PMID: 17435758, https://doi.org/10.1038/ng2026.

Durrant C, Mott R. 2010. Bayesian quantitative trait locus mapping using inferred haplotypes. Genetics 184(3):839–852, PMID: 20048050, https://doi.org/10.1534/genetics.109.113183.

Eduati F, Mangravite LM, Wang T, Tang H, Bare JC, Huang R, et al. 2015. Prediction of human population responses to toxic compounds by a collaborative competition. Nat Biotechnol 33(9):933–940, PMID: 26258538, https://doi.org/10.1038/nbt.3299.

Festing MF. 2010. Improving toxicity screening and drug development by using genetically defined strains. Methods Mol Biol 602:1–21, PMID: 20012389, https://doi.org/10.1007/978-1-60761-058-8_1.

Festing MF. 2014. Evidence should trump intuition by preferring inbred strains to outbred stocks in preclinical research. ILAR J 55(3):399–404, PMID: 25541542, https://doi.org/10.1093/ilar/ilu036.

Festing MF. 2016. Genetically defined strains in drug development and toxicity testing. Methods Mol Biol 1438:1–17, PMID: 27150081, https://doi.org/10.1007/978-1-4939-3661-8_1.

French JE, Gatti DM, Morgan DL, Kissling GE, Shockley KR, Knudsen GA, et al. 2015. Diversity Outbred mice identify population-based exposure thresholds and genetic factors that influence benzene-induced genotoxicity. Environ Health Perspect 123(3):237–245, PMID: 25376053, https://doi.org/10.1289/ehp.1408202.

Gatti DM, Broman K, Shabalin A. 2014a. DOQTL: genotyping and QTL mapping in DO mice. Version 1.12.0. Bioconductor. https://rdrr.io/bioc/DOQTL/ [accessed 25 October 2016].

Gatti DM, Svenson KL, Shabalin A, Wu LY, Valdar W, Simecek P, et al. 2014b. Quantitative trait locus mapping methods for Diversity Outbred mice. G3 (Bethesda) 4(9):1623–1633, PMID: 25237114, https://doi.org/10.1534/g3.114.013748.

Gauguier D, Froguel P, Parent V, Bernard C, Bihoreau MT, Portha B, et al. 1996. Chromosomal mapping of genetic loci associated with non-insulin dependent diabetes in the GK rat. Nat Genet 12(1):38–43, PMID: 8528248, https://doi.org/10.1038/ng0196-38.

Grubb SC, Bult CJ, Bogue MA. 2014. Mouse phenome database. Nucleic Acids Res 42(database issue):D825–D834, PMID: 24243846, https://doi.org/10.1093/nar/gkt1159.

Halliwell WH. 1997. Cationic amphiphilic drug-induced phospholipidosis. Toxicol Pathol 25(1):53–60, PMID: 9061852, https://doi.org/10.1177/019262339702500111.

Harrill AH, Desmet KD, Wolf KK, Bridges AS, Eaddy JS, Kurtz CL, et al. 2012. A mouse diversity panel approach reveals the potential for clinical kidney injury due to DB289 not predicted by classical rodent models. Toxicol Sci 130(2):416–426, PMID: 22940726, https://doi.org/10.1093/toxsci/kfs238.

Harrill AH, Ross PK, Gatti DM, Threadgill DW, Rusyn I. 2009a. Population-based discovery of toxicogenomics biomarkers for hepatotoxicity using a laboratory strain diversity panel. Toxicol Sci 110(1):235–243, PMID: 19420014, https://doi.org/10.1093/toxsci/kfp096.

Harrill AH, Watkins PB, Su S, Ross PK, Harbourt DE, Stylianou IM, et al. 2009b. Mouse population-guided resequencing reveals that variants in CD44 contribute to acetaminophen-induced liver injury in humans. Genome Res 19(9):1507–1515, PMID: 19416960, https://doi.org/10.1101/gr.090241.108.

Harrison DE, Astle CM, Niazi MK, Major S, Beamer GL. 2014. Genetically diverse mice are novel and valuable models of age-associated susceptibility to mycobacterium tuberculosis. Immun Ageing 11(1):24, PMID: 25606048, https://doi.org/10.1186/s12979-014-0024-6.

Hartman JH, Miller GP, Caro AA, Byrum SD, Orr LM, Mackintosh SG, et al. 2017. 1,3-Butadiene-induced mitochondrial dysfunction is correlated with mitochondrial CYP2E1 activity in Collaborative Cross mice. Toxicology 378:114–124, PMID: 28082109, https://doi.org/10.1016/j.tox.2017.01.005.

Iraqi FA, Athamni H, Dorman A, Salymah Y, Tomlinson I, Nashif A, et al. 2014. Heritability and coefficient of genetic variation analyses of phenotypic traits provide strong basis for high-resolution QTL mapping in the Collaborative Cross mouse genetic reference population. Mamm Genome 25(3–4):109–119, PMID: 24445421, https://doi.org/10.1007/s00335-014-9503-5.

Kang EY, Han B, Furlotte N, Joo JW, Shih D, Davis RC, et al. 2014. Meta-analysis identifies gene-by-environment interactions as demonstrated in a study of 4,965 mice. PLoS Genet 10(1):e1004022, https://doi.org/10.1371/journal.pgen.1004022.

Kang HM, Zaitlen NA, Wade CM, Kirby A, Heckerman D, Daly MJ, et al. 2008. Efficient control of population structure in model organism association mapping. Genetics 178(3):1709–1723, PMID: 18385116, https://doi.org/10.1534/genetics.107.080101.

Koturbash I, Scherhag A, Sorrentino J, Sexton K, Bodnar W, Swenberg JA, et al. 2011. Epigenetic mechanisms of mouse interstrain variability in genotoxicity of the environmental toxicant 1,3-butadiene. Toxicol Sci 122(2):448–456, PMID: 21602187, https://doi.org/10.1093/toxsci/kfr133.

Krewski D, Westphal M, Andersen ME, Paoli GM, Chiu WA, Al-Zoughool M et al. 2014. A framework for the next generation of risk science. Environ Health Perspect 122(8):796–805, PMID: 24727499, https://doi.org/10.1289/ehp.1307260.

Lan Q, Zhang L, Li G, Vermeulen R, Weinberg RS, Dosemeci M, et al. 2004. Hematotoxicity in workers exposed to low levels of benzene. Science 306(5702):1774–1776, PMID: 15576619, https://doi.org/10.1126/science.1102443.

Li D, Qiu Z, Shao Y, Chen Y, Guan Y, Liu M, et al. 2013. Heritable gene targeting in the mouse and rat using a CRISPR-Cas system. Nat Biotechnol 31(8):681–683, PMID: 23929336, https://doi.org/10.1038/nbt.2661.

Logan RW, Robledo RF, Recla JM, Philip VM, Bubier JA, Jay JJ, et al. 2013. High-precision genetic mapping of behavioral traits in the Diversity Outbred mouse population. Genes Brain Behav 12(4):424–437, PMID: 23433259, https://doi.org/10.1111/gbb.12029.

Lu L, Pandey AK, Houseal MT, Mulligan MK. 2016. The genetic architecture of murine glutathione transferases. PLoS One 11:e0148230, PMID: 26829228, https://doi.org/10.1371/journal.pone.0148230.

Lusis AJ, Seldin MM, Allayee H, Bennett BJ, Civelek M, Davis RC, et al. 2016. The Hybrid Mouse Diversity Panel: a resource for systems genetics analyses of metabolic and cardiovascular traits. J Lipid Res 57(6):925–942, PMID: 27099397, https://doi.org/10.1194/jlr.R066944.

Mallal S, Phillips E, Carosi G, Molina JM, Workman C, Tomazic J, et al. 2008. HLA-B*5701 screening for hypersensitivity to abacavir. N Engl J Med 358(6):568–579, PMID: 18256392, https://doi.org/10.1056/NEJMoa0706135.

Manolio TA, Brooks LD, Collins FS. 2008. A HapMap harvest of insights into the genetics of common disease. J Clin Invest 118(5):1590–1605, PMID: 18451988, https://doi.org/10.1172/JCI34772.

McBride WJ, Rodd ZA, Bell RL, Lumeng L, Li TK. 2014. The alcohol-preferring (P) and high-alcohol-drinking (HAD) rats–animal models of alcoholism. Alcohol 48(3):209–215, PMID: 24268381, https://doi.org/10.1016/j.alcohol.2013.09.044.

Mosedale M, Wu H, Kurtz CL, Schmidt SP, Adkins K, Harrill AH. 2014. Dysregulation of protein degradation pathways may mediate the liver injury and phospholipidosis associated with a cationic amphiphilic antibiotic drug. Toxicol Appl Pharmacol 280(1):21–29, PMID: 24967691, https://doi.org/10.1016/j.taap.2014.06.013.

Nachshon A, Abu-Toamih Atamni HJ, Steuerman Y, Sheikh-Hamed R, Dorman A, Mott R, et al. 2016. Dissecting the effect of genetic variation on the hepatic expression of drug disposition genes across the Collaborative Cross mouse strains. Front Genet 7:172, PMID: 27761138, https://doi.org/10.3389/fgene.2016.00172.

National Academies of Sciences and Medicine. 2012. *Exposure Science in the 21st Century: A Vision and a Strategy*. Washington, DC:National Academies Press.

National Academies of Sciences and Medicine. 2017. *Using 21st Century Science to Improve Risk-Related Evaluations*. Washington, DC:National Academies Press.

Navarro VJ, Bonkovsky HL, Hwang SI, Vega M, Barnhart H, Serrano J. 2013. Catechins in dietary supplements and hepatotoxicity. Dig Dis Sci 58(9):2682–2690, PMID: 23625293, https://doi.org/10.1007/s10620-013-2687-9.

Nguyen HT, Tsuchiya MC, Yoo J, Iida M, Agusa T, Hirano M, et al. 2016. Strain differences in the proteome of dioxin-sensitive and dioxin-resistant mice treated with 2,3,7,8-tetrabromodibenzo-p-dioxin. Arch Toxicol 91(4):1763–1782, PMID: 27604104, https://doi.org/10.1007/s00204-016-1834-4.

Pan F, McMillan L, Pardo-Manuel D, Villena F, Threadgill D, Wang W. 2009. TreeQA: quantitative genome wide association mapping using local perfect phylogeny trees. Pac Symp Biocomput 2009:415–426, PMID: 19209719.

Papanicolaou KN, Khairallah RJ, Ngoh GA, Chikando A, Luptak I, O'Shea KM, et al. 2011. Mitofusin-2 maintains mitochondrial structure and contributes to stress-induced permeability transition in cardiac myocytes. Mol Cell Biol 31(6):1309–1328, PMID: 21245373, https://doi.org/10.1128/MCB.00911-10.

Philip VM, Sokoloff G, Ackert-Bicknell CL, Striz M, Branstetter L, Beckmann MA, et al. 2011. Genetic analysis in the Collaborative Cross breeding population. Genome Res 21(8):1223–1238, PMID: 21734011, https://doi.org/10.1101/gr.113886.110.

Rasmussen AL, Okumura A, Ferris MT, Green R, Feldmann F, Kelly SM, et al. 2014. Host genetic diversity enables Ebola hemorrhagic fever pathogenesis and resistance. Science 346(6212):987–991, PMID: 25359852, https://doi.org/10.1126/science.1259595.

Recla JM, Robledo RF, Gatti DM, Bult CJ, Churchill GA, Chesler EJ. 2014. Precise genetic mapping and integrative bioinformatics in Diversity Outbred mice reveals *Hydin* as a novel pain gene. Mamm Genome 25(5–6):211–222, PMID: 24700285, https://doi.org/10.1007/s00335-014-9508-0.

Rockman MV, Kruglyak L. 2008. Breeding designs for recombinant inbred advanced intercross lines. Genetics 179(2):1069–1078, PMID: 18505881, https://doi.org/10.1534/genetics.107.083873.

Rogala AR, Morgan AP, Christensen AM, Gooch TJ, Bell TA, Miller DR, et al. 2014. The Collaborative Cross as a resource for modeling human disease: CC011/Unc, a new mouse model for spontaneous colitis. Mamm Genome 25(3–4):95–108, PMID: 24487921, https://doi.org/10.1007/s00335-013-9499-2.

Shimoyama M, De Pons J, Hayman GT, Laulederkind SJ, Liu W, Nigam R, et al. 2015. The Rat Genome Database 2015: genomic, phenotypic and environmental variations and disease. Nucleic Acids Res 43(database issue):D743–D750, PMID: 25355511, https://doi.org/10.1093/nar/gku1026.

Smallwood TL, Gatti DM, Quizon P, Weinstock GM, Jung KC, Zhao L, et al. 2014. High-resolution genetic mapping in the Diversity Outbred mouse population identifies *Apobec1* as a candidate gene for atherosclerosis. G3 (Bethesda) 4(12):2353–2363, PMID: 25344410, https://doi.org/10.1534/g3.114.014704.

Solberg Woods LC. 2014. QTL mapping in outbred populations: successes and challenges. Physiol Genomics 46(3):81–90, PMID: 24326347, https://doi.org/10.1152/physiolgenomics.00127.2013.

STAR Consortium, Saar K, Beck A, Bihoreau MT, Birney E, Brocklebank D, et al. 2008. SNP and haplotype mapping for genetic analysis in the rat. Nat Genet 40(5):560–566, PMID: 18443594, https://doi.org/10.1038/ng.124.

Suzuki OT, Frick A, Parks BB, Trask OJ Jr, Butz N, Steffy B, et al. 2014. A cellular genetics approach identifies gene-drug interactions and pinpoints drug toxicity pathway nodes. Front Genet 5:272, PMID: 25221565, https://doi.org/10.3389/fgene.2014.00272.

Threadgill DW, Hunter KW, Williams RW. 2002. Genetic dissection of complex and quantitative traits: from fantasy to reality via a community effort. Mamm Genome 13(4):175–178, PMID: 11956758, https://doi.org/10.1007/s00335-001-4001-Y.

Tsaih SW, Lu L, Airey DC, Williams RW, Churchill GA. 2005. Quantitative trait mapping in a diallel cross of recombinant inbred lines. Mamm Genome 16(5):344–355, PMID: 16104382.

U.S. EPA (U.S. Environmental Protection Agency). 1992. Guidelines for Exposure Assessment. https://www.epa.gov/risk/guidelines-exposure-assessment [accessed 10 February 2017].

U.S. EPA. 2014. Guidance for Applying Quantitative Data to Develop Data-Derived Extrapolation Factors for Interspecies and Intraspecies Extrapolation. EPA/100/R-14/002F. https://www.epa.gov/risk/guidance-applying-quantitative-data-develop-data-derived-extrapolation-factors-interspecies-and [accessed 8 February 2017.

Vanderlinden LA, Saba LM, Printz MP, Flodman P, Koob G, Richardson HN, et al. 2014. Is the alcohol deprivation effect genetically mediated? Studies with HXB/BXH recombinant inbred rat strains. Alcohol Clin Exp Res 38(7):2148–2157, PMID: 24961585, https://doi.org/10.1111/acer.12471.

Venter JC, Adams MD, Myers EW, Li PW, Mural RJ, Sutton GG, et al. 2001. The sequence of the human genome. Science 291:1304–1351, PMID: 11181995, https://doi.org/10.1126/science.1058040.

Vered K, Durrant C, Mott R, Iraqi FA. 2014. Susceptibility to *Klebsiella pneumonaie* infection in collaborative cross mice is a complex trait controlled by at least three loci acting at different time points. BMC genomics 15:865, PMID: 25283706, https://doi.org/10.1186/1471-2164-15-865.

Welsh CE, Miller DR, Manly KF, Wang J, McMillan L, Morahan G, et al. 2012. Status and access to the Collaborative Cross population. Mamm Genome 23(9–10):706–712, PMID: 22847377, https://doi.org/10.1007/s00335-012-9410-6.

Weng Z, Zhou P, Salminen WF, Yang X, Harrill AH, Cao Z, et al. 2014. Green tea epigallocatechin gallate binds to and inhibits respiratory complexes in swelling but not normal rat hepatic mitochondria. Biochem Biophys Res Commun 443(3):1097–1104, PMID: 24384371, https://doi.org/10.1016/j.bbrc.2013.12.110.

Wiles MV, Taft RA. 2010. The sophisticated mouse: protecting a precious reagent. Methods Mol Biol 602:23–36, PMID: 20012390, https://doi.org/10.1007/978-1-60761-058-8_2.

Yang H, Bell TA, Churchill GA, Pardo-Manuel de Villena F. 2007. On the subspecific origin of the laboratory mouse. Nat Genet 39(9):1100–1107, PMID: 17660819, https://doi.org/10.1038/ng2087.

Yang H, Ding Y, Hutchins LN, Szatkiewicz J, Bell TA, Paigen BJ, et al. 2009. A customized and versatile high-density genotyping array for the mouse. Nat Methods 6(9):663–666, PMID: 19668205, https://doi.org/10.1038/nmeth.1359.

Yang H, Wang JR, Didion JP, Buus RJ, Bell TA, Welsh CE, et al. 2011. Subspecific origin and haplotype diversity in the laboratory mouse. Nat Genet 43(7):648–655, PMID: 21623374, https://doi.org/10.1038/ng.847.

Yoo HS, Bradford BU, Kosyk O, Uehara T, Shymonyak S, Collins LB, et al. 2015. Comparative analysis of the relationship between trichloroethylene metabolism and tissue-specific toxicity among inbred mouse strains: kidney effects. J Toxicol Environ Health Part A 78(1):32–49, PMID: 25424545, https://doi.org/10.1080/15287394.2015.958418.

Zeise L, Bois FY, Chiu WA, Hattis D, Rusyn I, Guyton KZ. 2013. Addressing human variability in next-generation human health risk assessments of environmental chemicals. Environ Health Perspect 121(1):23–31, PMID: 23086705, https://doi.org/10.1289/ehp.1205687.

Zou F, Gelfond JA, Airey DC, Lu L, Manly KF, Williams RW, et al. 2005. Quantitative trait locus analysis using recombinant inbred intercrosses: theoretical and empirical considerations. Genetics 170(3):1299–1311, PMID: 15879512, https://doi.org/10.1534/genetics.104.035709.